Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the in-house investigations of **Rennet** in the developing chicken embryo 10/23/75

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
OFFICE OF THE SECRETARY

TO : GRAS Review Branch, HFF-335

DATE: October 23, 1975

THRU : Dr. Herbert Blumenthal, Acting Director
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph.D. M. Jacqueline Verrett
Reproductive Physiology Branch, HFF-157

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substance to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Rennet in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Rennet

Protocol:

Rennet (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Air cell treatment at 0 hours showed no toxicity above background. When administered via the air cell at 96 hours there was a regression of mortality on dose with a calculated LD_{50} of 85.24 mg/kg (4.26 mg/egg). Yolk treatment at both times resulted in a regression line whose slope was not significantly different from zero ($p = 0.05$).

Scattered abnormalities were observed for all four conditions of test, but in no instances were these significantly higher than or different from those observed in the solvent-treated or untreated control eggs. Rennet displayed no teratogenicity under the test conditions employed.

1. Rennet, Miles Laboratories, Elkhart, Indiana.

2. McLaughlin, J., Jr., Marliac, J. P., Verret, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770

3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006

4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Rennet
Air Cell @ 0 Hours

Table 1

Dose		Number of	**Percent	Percent Abnormal	
mg/egg	mg/kg	Eggs	Mortality	Total	Structural
10.00	200.00	140	34.28*	2.14	0.71
5.00	100.00	140	34.28*	2.14	0.00
2.50	50.00	140	35.00*	3.57	1.42
1.250	25.00	140	32.85*	1.42	0.00
0.500	10.00	139	28.77*	1.43	0.00
Water	--	174	16.66	0.57	0.57
Controls	--	548	10.58	0.91	0.54

*Significantly different from solvent $p \leq 0.05$
**Slope is not significantly different from zero $p = 0.05$

Rennet
Air Cell @ 96 Hours

Table2

Dose		Number of	**Percent	Percent Abnormal	
mg/egg	mg/kg	Eggs	Mortality	Total	Structural
5.00	100.00	110	77.27*	7.27	6.36
2.50	50.00	110	21.81	1.81	0.00
1.250	25.00	110	12.72*	0.90	0.00
0.6250	12.50	109	10.09*	0.91	0.90
0.250	5.00	110	17.27	0.90	0.90
Water	--	130	26.15	0.76	0.76
Controls	--	548	10.58	0.91	0.54

*Significantly different from solvent $p \leq 0.05$
**LD_{50} 85.2430 mg/kg (4.262 mg/egg)

Rennet
Yolk @ 0 Hours

Table 3

Dose		Number of	**Percent	Percent Abnormal	
mg/egg	mg/kg	Eggs	Mortality	Total	Structural
10.00	200.00	80	90.00*	0.00	0.00
5.00	100.00	80	86.25*	1.25	0.00
2.50	50.00	80	91.25*	0.00	0.00
1.250	25.00	80	88.75*	1.25	0.00
0.500	10.00	80	85.00*	0.00	0.00
Water	--	105	46.66	0.00	0.00
Controls	--	548	10.58	0.91	0.54

*Significantly different from solvent $p \leq 0.05$
**Slope not significantly different from zero $p = 0.05$

Rennet
Yolk @ 96 Hours

Table 4

Dose		Number of	**Percent	Percent Abnormal	
mg/egg	mg/kg	Eggs	Mortality	Total	Structural
5.00	100.00	110	80.00*	4.54*	0.90
2.50	50.00	110	59.09*	3.63	1.81
1.250	25.00	110	72.72*	0.90	0.90
0.6250	12.50	110	71.81*	5.45	5.45
0.250	5.00	110	60.90*	2.72	0.90
Water	--	100	42.00	0.00	0.00
Controls	--	548	10.58	0.91	0.54

*Significantly different from solvent $p \leq 0.05$
**Slope not significantly different from zero $p = 0.05$